

## WHAT IS CLAIMED IS:

1        1. A method for purification, modification and immobilization of recombinant protein,  
2        said method comprising the steps of:  
3                tagging a DNA sequence encoding a target protein into a recombinant vector with  
4        a specific tag sequence;  
5                expressing the vector under suitable condition to obtain a recombinant protein;  
6                purifying and modifying said recombinant protein by using an affinity column and  
7        a modification reagent;  
8                exchanging said recombinant protein which has been attached to the affinity  
9        column with a decoupling reagent; and  
10        immobilizing said recombinant protein onto a substrate.

1        2. The method as claimed in claim 1, wherein said specific tag comprises Histidine  
2        tag, Maltose-binding tag, or GST tag.

1        3. The method as claimed in claim 2, wherein said specific genetic tag is Histidine  
2        tag.

1        4. The method as claimed in claim 1, wherein said recombinant protein is prepared  
2        by using prokaryotic cell, eukaryotic cell or an *in vitro* transcription/translation system.

3        5. The method as claimed in claim 4, wherein said prokaryotic cell is *E. coli*.

4        6. The method as claimed in claim 4, wherein said eukaryotic cell is yeast, insect  
5        cell or mammalian cell.

6        7. The method as claimed in claim 1, wherein the affinity column for capturing the  
7        recombinant protein is chosen in corresponding to said specific tag.

1        8. The method as claimed in claim 7, when said specific tag is Histidine tag, a metal  
2        chelating column is used as the affinity column.

1           9. The method as claimed in claim 8, wherein the metal chelation column is  
2 represented by a general formula as metal-X column.

1           10. The method as claimed in claim 9, wherein the metal in said formula comprises  
2 nickel, zinc, copper, or cobalt.

1           11. The method as claimed in claim 9, wherein the X in said formula comprises  
2 iminodiacetic acid, nitrilotriacetic acid, tris(carboxymethyl)ethylendiamin,  
3 carboxymethylaspartate, or TALON.

1           12. The method as claimed in claim 9, wherein the metal-X column is Ni-IDA  
2 column or Cu-IDA column.

1           13. The method as claimed in claim 7, when said specific tag is Maltose-binding tag,  
2 an amylose column is used as the affinity column.

1           14. The method as claimed in claim 7, when said specific tag is a GST-tag,  
2 glutathione column is used as the affinity column.

1           15. The method as claimed in claim 1, wherein said recombinant protein is  
2 modified by using a biotinylation reaction so to add biotin functional groups to said  
3 recombinant protein.

1           16. The method as claimed in claim 15, wherein the modification of said  
2 recombinant protein comprising the steps of:

3           obtaining a solution containing the recombinant protein;

4           adding a biotinylation reagent to cause biotinylation reaction with said recombinant  
5 protein; and

6 capturing said biotinlyted recombinant protein by using the affinity column so as to  
7 fixate said biotinlyted recombinant protein in said affinity column.

1 17. The method as claimed in claim 15, wherein the modification of said  
2 recombinant protein comprising the steps of:

3 obtaining a solution containing the recombinant protein;

4 capturing said recombinant protein by using the affinity coloum so as to fixate said  
5 recombinant protein in said affinity column; and

6 adding a biotinylation reagent to said affinity column to cause biotinylation  
7 reaction with said recombinant protein fixated in said affinity column.

1 18. The method as claimed in claim 16, wherein said recombinant protein is  
2 exchanged from the affinity column by a decoupling reagent, said decoupling reagent is  
3 chosen according to the properties of the specific tag and the affinity column.

1 19. The method as claimed in claim 18, when said specific tag is Histidine tag and  
2 the affinity column is a metal chelating column, the decoupling reagent is immidazole.

1 20. The method as claimed in claim 18, when said specific tag is maltose-binding  
2 tag and the affinity column is an amylose column, the decoupling reagent is maltose.

1 21. The method as claimed in claim 18, when said specific tag is GST tag and the  
2 affinity column is a glutathione column, the decoupling reagent is glutathione.

1 22. The method as claimed in claim 1, wherein the immobilization of said  
2 recombinant protein is achieved by modifying the recombinant protein with biotin and  
3 attaching the biotin-modified recombinant protien on a sub̄strate coated with streptavidin.

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